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New Twists in Gene Regulation by Glucocorticoid Receptor: Is DNA Binding Dispensable?

Minireview

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Discovered in the 1930s in adrenal gland extracts, glucocorticoid hormones (GCs) were amongst the first steroid hormones to be found. The study of GC action provided many important and critical insights to the mechanisms used by nuclear receptors and other sequence-specific transcription factors to regulate gene transcription. During the 1970s it was realized that GCs exert their biological effects through a specific receptor, GR, a DNA-binding protein whose nuclear translocation is induced upon ligand binding (Yamamoto, 1985; Beato et al., 1995). GCs were found to induce transcription of target genes, the most immediate being those whose induction is insensitive to inhibitors of protein synthesis. Cloning and analysis of such genes, the murine mammary tumor virus (MMTV) genome and the human metallothionein II_A ($hMTII_A$) gene, led to identification of the first hormone response elements (HREs), in this case called GREs, which serve as inducible enhancer elements and GR-binding sites (reviewed by Yamamoto, 1985; Beato et al., 1995). These findings obtained in the early 1980s forged the notion that GR is a sequence-specific ligand-regulated transcription factor, one of the first eukaryotic transcription factors to be identified. This model was considered proven when the GR was molecularly cloned and the mechanism by which it activates transcription of GRE-containing genes was studied in detail (reviewed by Beato et al., 1995). It was even considered that all of the physiological effects of GC are mediated through gene induction (Yamamoto, 1985). Results published in this issue of *Cell* (Reichardt et al., 1998) question whether DNA binding is required at all for many of the physiological functions of GR.

Distinct Modes of Transcriptional Control

The GR, a prototypical nuclear receptor, is composed of three functional domains: a constitutive N-terminal activation domain (AF1 or τ1), a central DNA-binding domain (DBD), and a C-terminal ligand-dependent activation function (AF2 or τ2), a part of the ligand-binding domain (Beato et al., 1995). Mutational analysis of GREs revealed them to be palindromic sequences composed of 6 bp half-sites separated by a 3 bp spacer (Beato et al., 1995). This organization suggests that GR binds such sites as a dimer. However, it is important to realize that the major form of the liganded receptor is monomeric in solution and that dimerization occurs only after binding to palindromic GREs, accounting for cooperative DNA binding (Dahlman-Wright et al., 1991; Luisi et al., 1991). Nevertheless, structural analysis of GR–GRE complexes reveals that the D loop within the DBD is responsible for postbinding dimerization (Luisi et al., 1991). Mutations within this region have a devastating effect on cooperative binding to palindromic GREs, but do not completely abolish DNA binding (Dahlman-Wright et al., 1991).

The view of GR as a ligand-regulated transcription factor that accomplishes all of its physiological functions by binding palindromic GREs and inducing expression of genes that contain such elements, however, lasted only until 1990. Although it was realized that GC can also repress the expression of certain genes, most of the attention until that time has focused on positively regulated genes. However, in the late 1980s several groups began to analyze genes that are negatively regulated by GC. The first genes subjected to such scrutiny included those coding for proopiomelanocortin (POMC), the α subunit of glycoprotein hormones, and collagenase type I. These genes represent important physiological targets for GC.

In the case of the α subunit gene GC-mediated repression is caused by interference with the cAMP-responsive transcription factor CREB (Akerblom et al., 1988), while repression of collagenase transcription was attributed to GR-mediated interference with transcription factor AP-1 (Jonat et al., 1990; Yang-Yen et al., 1990). AP-1 activity is stimulated by many agonists of which proinflammatory cytokines are most relevant in this context. Negative regulation of the POMC gene, on the other hand, is exerted through direct interaction of GR with its promoter, which contains a negative GRE (nGRE; Drouin et al., 1993). Whereas the exact mechanism by which GR interferes with CREB or AP-1 activity is yet to be elucidated, it is almost certain that it does not require direct binding to DNA. Mutations within the DBD or substitutions of the GR DBD with DBDs of other transcription factors do not abolish GR's ability to interfere with AP-1 (Jonat et al., 1990; Yang-Yen et al., 1990). Importantly, a dimerization-defective GR, generated by a mutation within the D loop (A458T), does not bind cooperatively to GREs but can still repress AP-1 regulated genes (Heck et al., 1994).

Another transcription factor whose activity is negatively regulated by GC is NF-κB. While transient transfection experiments suggest that this case of negative regulation is also based on direct interaction between GR and the affected transcription factor, other experiments, some of which were conducted in vivo, indicate that at least in lymphoid cells the inhibition of NF-κB activity by GC is based on induction of IκBα, a specific NF-κB inhibitor (discussed in Auphan et al., 1995). Although GC stimulate IκBα gene transcription, the GRE responsible for this effect has not been identified, so IκBα induction and NF-κB inhibition may not depend on direct binding of GR to DNA. In summary, there are at least three major modes through which GC can regulate gene transcription: (1) activation via binding of GR to positive GREs; (2) repression via binding of GR to nGREs; and (3) transcriptional interference via interactions of GR with other transcription factors (Figure 1).

Physiological and Pharmacological Actions of GC

An extremely important question is which of the many biological activities of GC are mediated through each

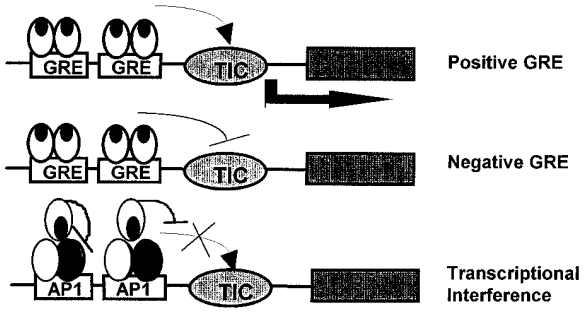

Figure 1. Mechanism of Transcriptional Regulation by Glucocorticoids

Typical GR and AP-1 regulated promoters are illustrated as containing two GREs or AP-1 sites, although the actual number of binding sites can vary from one to several. Interference with AP-1 activity could be mediated through a direct effect on some aspect of AP-1 function or through tethering of GR to AP-1 followed by an interaction with coactivators or components of the transcriptional initiation complex (TIC).

of these three mechanisms. Before trying to address this question, it is necessary to review briefly GC physiology and pharmacology. Most illuminating in this respect are the symptoms of primary adrenocortical insufficiency, first described by Addison in the mid nineteenth century as "general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach and a peculiar change in the colour of the skin" (Felig et al., 1987). As defined by studies of human patients and adrenalectomized animals, the most important actions of GC include stimulation of gluconeogenesis, decreased glucose uptake and utilization in peripheral tissues, increased glycogen deposition, increased lipolysis and free fatty acid release, stimulation of protein and nucleic acid synthesis especially in liver, suppression of immune and inflammatory responses, inhibition of cytokine synthesis, inhibition of fibroblast proliferation and extracellular matrix deposition, maintenance of normal cardiovascular function and blood pressure, regulation of calcium absorption and redistribution, acceleration of various developmental events, and tissue maturation, most notably the liver, pancreas, GI track, and lung (Felig et al., 1987).

While these are the general, day-to-day functions of GC, these hormones are especially important for combating stress. GC synthesis and release are elevated during stress leading to increased ventricular work load; inhibition of inflammatory mediator and cytokine synthesis; inhibition of vasodilator production; increased glucose production; induction of glutamine synthase in the brain, providing protection from the excitotoxic amino acid glutamate; and induction of metallothionein in the liver, resulting in chelation of toxic heavy metals (Felig et al., 1987). As summarized succinctly by Addison, insufficient production of GC results in weakness, weight loss, anorexia, gastrointestinal symptoms, such as nausea, abdominal discomfort and vomiting, hyperpigmentation, and hypotension. While none of these symptoms is life threatening, patients suffering from adrenocortical insufficiency are unable to withstand stress and can suffer an acute adrenal crisis, a critical syndrome induced by stress. Acute adrenal crisis involves profound anorexia, nausea, vomiting to the point of dehydration, and a severe drop in blood pressure leading to shock or coma (Felig et al., 1987).

There are two major pharmacological uses for GC. The most obvious one is treatment of adrenocortical insufficiencies. However GC are more commonly employed as anti-inflammatory and immunosuppressive drugs, due to their multiple inhibitory effects on the immune system. In young mice or rats, administration of GC causes thymic involution by inducing the apoptosis of double positive T cells. However, peripheral lymphocytes of both humans and rodents are much less sensitive to GC-induced lysis and the thymus is not an adult organ. Hence, the far more important anti-inflammatory and immunosuppressive activities of GC are the inhibition of cytokine gene induction, inhibition of chemokine synthesis, and repression of genes encoding cell surface receptors and adhesion molecules involved in lymphocyte activation, migration, and recruitment. As most of the immunoregulatory genes that are negatively regulated by GC are positively regulated by AP-1 and NF-κB, it is quite likely that the immunosuppressive and anti-inflammatory activities of GC are mediated through interference with these transcription factors (Barnes and Karin, 1997). By contrast, GC-induced T cell lysis is sensitive to inhibition of protein synthesis and therefore may depend on gene activation rather than gene repression.

Although GC are amongst the most potent immunosuppressive and anti-inflammatory drugs currently available, and are especially efficacious for treatment of diseases like asthma and rheumatoid arthritis, there are two major problems associated with their prolonged use. One is their metabolic side effects caused by alterations of glucose and lipid metabolism, which can result in hyperglycemia and decreased carbohydrate tolerance. The second problem is due to decreased collagen and extracellular matrix production by fibroblasts and enhanced bone resorption, resulting in osteoporosis (Felig et al., 1987). The first problem is caused by the gluconeogenic activity of GC and therefore depends on induction of genes such as those for tyrosine aminotransferase (TAT), alanine aminotransferase, and phosphoenolpyruvate carboxykinase (PEPCK). The regulatory regions of these genes contain positive GREs (Beato et al., 1995). However, the transcriptional mechanism underlying the osteolytic activity of GC remains to be identified. Understanding which transcriptional mechanism underlies each of the physiological and pharmacological activities of GC is of great importance, as it may enable the development of novel GC derivatives that will be able to promote one activity more efficiently than others and thus display increased therapeutic value and specificity. For instance, a novel GC was recently found that is more effective in promoting interference with AP-1 activity than GRE-mediated transactivation (Vayssiére et al., 1997). This and similar compounds may turn out to be better anti-inflammatory drugs than the standard GC currently in use.

Linking Molecular Mechanisms to Physiological Functions

Given the pleiotropic functions of GC, the plethora of molecular mechanisms through which they regulate

gene transcription, and their extensive clinical use and importance, it is of great interest and relevance to identify the molecular basis for each of the actions described above. A clever new approach to this important problem is described by Reichardt et al. (1998). Using the latest techniques in reversed mouse genetics, the authors have "knocked-in" a point mutation into the *GR* gene leading to substitution of alanine 458 by a threonine. As described above this substitution results in a dimerization-defective GR (GR^{dim}) that no longer binds cooperatively to palindromic GREs (Heck et al., 1994). Using liver nuclear extracts, Reichardt et al. verify that $GR^{dim/dim}$ mice no longer express palindromic GRE binding activity. However, they do not exclude the possibility that GR^{dim} can still bind to GRE halfsites. Nevertheless, using transiently transfected embryonic fibroblast cell lines and RNA prepared from livers of untreated and GC-injected mice, they do show that $GR^{dim/dim}$ mice no longer respond to GC with induction of TAT mRNA (Reichardt et al., 1998). In addition, there is a large reduction in the inducibility of an MMTV-based reporter. The residual induction is probably due to the fact that GR^{dim} can bind noncooperatively to the multiple GREs present in the MMTV promoter. Given that these are typical GC-inducible genes, it is safe to assume that $GR^{dim/dim}$ mice are defective by-and-large in mounting a positive transcriptional response to GC. However, some genes containing multiple GREs may be weakly induced, and this could be physiologically relevant.

As $GR^{dim/dim}$ mice appear relatively normal and healthy under standard laboratory conditions, the most striking outcome of these experiments is that GRE-mediated gene activation is not necessary for development and survival. These results are surprising because the same group had previously generated GR-deficient mice ($GR^{-/-}$) by inactivating the *GR* gene (Cole et al., 1995). Unlike the $GR^{dim/dim}$ mice, $GR^{-/-}$ mice die shortly after birth due to respiratory failure because their lungs have not matured, lacking surfactant proteins. Clearly, GRE-mediated gene induction is not the basis for GC-induced lung maturation. Although GC are used clinically to induce lung maturation and surfactant expression in human fetuses expected to be delivered prematurely, no functional GREs have been described in the promoter region of any surfactant gene. A common defect for $GR^{-/-}$ and $GR^{dim/dim}$ mice is the failure to express gluconeogenic enzymes. While these results indicate that expression of these enzymes is not necessary for viability under standard laboratory conditions, the lesson learned from human patients suffering from acute adrenal crisis suggests that induction of gluconeogenic enzymes may be required for survival under stress. However, the $GR^{dim/dim}$ mice have not yet been subjected to a stress test to examine this important point.

In addition to a defect in GRE-mediated gene induction, the $GR^{dim/dim}$ mice are defective in repression of genes that are regulated through nGREs, exhibiting elevated expression of POMC and prolactin, two genes that are controlled by nGREs (Sakai et al., 1988; Drouin et al., 1993). The only transcriptional regulatory function that is clearly intact in $GR^{dim/dim}$ mice is transcriptional interference. Reichardt et al. demonstrate that interference with AP-1 activity and repression of AP-1-regulated genes, like collagenase and gelatinase, are not affected in cells isolated from $GR^{dim/dim}$ mice. However, the authors have yet to examine this interesting mutant for regulation of NF-κB activity and repression of NF-κB dependent target genes.

Given that transcriptional interference is the only regulatory function that remains intact in $GR^{dim/dim}$ mice, the obvious question that arises is which of the physiological and pharmacological actions of GC are mediated through this mechanism. However, the curious reader will have to wait until the $GR^{dim/dim}$ mice are subjected to more tests and tribulations. For instance, it is likely that the anti-inflammatory and immunosuppressive activity of GC is mostly dependent on interference with AP-1 and NF-κB. Yet the only immune function examined in the $GR^{dim/dim}$ mice is GC-induced thymic involution. Clearly, $GR^{dim/dim}$ mice are fully resistant to GC-induced killing of double positive thymocytes, and thus this killing must not necessitate interference with AP-1 activity, but rather is likely to relie on binding of GR to GREs. However, until we know how NF-κB activity is affected in $GR^{dim/dim}$ mice, we will have to refrain from judging whether repression of NF-κB activity is a major contributor to thymocyte killing by GC. In contrast with thymic involution, GC-mediated killing of human lymphomas, which represents another therapeutic use for GC, does not seem to require the activating function of GR (Helmberg et al., 1995) and may depend on repression of NF-κB-induced survival factors (Beg and Baltimore, 1996; Liu et al., 1996). Also, the feedback inhibition of GC secretion mediated via inhibition of corticotropin releasing factor synthesis in the hypothalamus is most likley based on transcriptional interference rather than binding of GR to nGREs, since the circulating levels of GC are normal in the $GR^{dim/dim}$ mice (Reichardt et al., 1998). The most interesting question, at least for clinicians and drug developers, is whether GC administration to $GR^{dim/dim}$ mice will result in repression of genes coding for cytokines, chemokines, adhesion molecules, and enzymes that produce inflammatory mediators. It is the repression of such genes that accounts for most of the anti-inflammatory and immunosuppressive activities of GC. Also of great interest would be how $GR^{dim/dim}$ mice respond to various stresses and whether they display the same drop in blood pressure as exhibited by patients suffering from adrenal crisis. As the ability of GC to restore blood pressure in such patients is most likely mediated through inhibition of prostaglandin and leukotriene production (probably through inhibition of cyclooxygenase 2 induction, which is likely to be mediated by interference with AP-1 or NF-κB), it can be expected that as long as GC will exhibit anti-inflammatory activity in $GR^{dim/dim}$ mice, GC should also be able to maintain blood pressure. Thus, $GR^{dim/dim}$ mice may be able to survive acute stress.

Although more experiments need to be performed with these valuable mutant mice before one could reach a sweeping conclusion, I find it difficult to refrain from speculation: if $GR^{dim/dim}$ mice can survive stress, maybe the most important functions of GC are not mediated at all through binding of GR to GREs. If this is indeed the case, maybe the most important function of GR is to regulate negatively the activities of AP-1 and NF-κB, two transcription factors whose involvement in inflammatory responses is evolutionary conserved between

mammals and insects (Meister et al., 1997). Thus, it is entirely possible that GC and GR have evolved as negative regulatory devices that prevent overstimulation and misfiring of the innate immune system. If this is true, which function, DNA binding or transcriptional interference, evolved first?

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